September 8, 1953

Dear Harriett:

Your very nice letter of the 3d arrived a short while ago— Esther and I were very pleased to hear from you and Boris. We are sorry to have been unable to make the meetings. I can assure you that the assumptions you described as gratuitous are not only this, but wrong. I don't know how everyone else seems to manage it, but we found that the expenses of a trip would have been beyond our means, especially as we are trying to conserve our savings for the usual bourgeois aim of buying a house. Had the invitations to the Congresses been accompanied by a pair of airplane tickets— well it would have been another story. Originally there had been an outside chance of official (Army) transportation, but this fell through; the University here has nothing to offer in travel gratuities, and the NSF grants were the insubstantial sum of $300. For possible future occasions, need I drop hints any stronger?

I don't know why there needs to be any excitement about the existence of a controversy on K-12 genetics. The facts will tell, in the long run, and possibly somewhat sooner if there is a little restraint on the injection of personalities into scientific issues. Many of the differences are, of course, semantic (as was recognized by the authors of the new bacterial cybernetics) and it is likely to turn out that we have, after all, been all talking about the same things in different language. One might well add that the correspondence of cybernetics to genetics is not unique to bacteriology, viz. (e.g.) Kalmus' little article in the Journal of Heredity, January 1950. Or more pertinent to the moment (and in a somewhat biblical tone) the information involved in mating of E. coli (interbacterial information) or other organisms (interDrosophilary information, interNeurospsporal information, or interSchizosaccharomycetological information) has to do with carnal knowledge.

Harriett: set your conscience at rest. Someone or other (probably Karl P. Link, who has a habit of sending me postcards dated 5 A.M. when he sees my name in print, to tell me about it) brought that Nature article to my attention, and we all had a good laugh. Two speculations only were excited: whether the occasion was good beer or bad absinthe, and (seeing not seeing your own name) whether the inter-Ephrussial information had broken down. The only thing that troubles me now is that you should have so dour a recollection of my own sense of humor as to think an explanation necessary. I would indeed like to have an autographed copy, preferably if the entire authorship is represented (though I doubt if there would be room on the reprint).

To go back to cybernetics, do you think there is any substantial contribution from this new fad to our own problems? I have not seen it.

I will protest at two disputable statements in the little paper, and in your letter: the first is that I am willing to accept responsibility for a somewhat excessive neologia: these are experiments that need not be taken too seriously. Some of these words will be discarded; others perhaps retained. In writing a paper, these semantic symbols have the same role for me that the terms defined in a mathematical discourse. If familiar words are used this way, there is the danger that the reader will not heed the specific definitions given to them. A new construction warns that the concept is not yet delimited by an existing, unam-

biguous and concise expression. But while my culpability in this respect may be more than average, I yield to Lwoff as the most accomplished master of this art. Secondly, I am not myself quite willing to admit that "transduction" and the K-12 sexual process are different aspects of a single phenomenon (in the same sense that you deny a bridge between classical genetics and the pneumococcus transformation), except in the most general sense that they effect genetic recombination [which is perhaps all that was meant].

Bill Hayes visited us just this last weekend— a personal meeting to which I have looked forward, and enjoyed very much. I will not pretend that we reached a complete agreement on every issue-- the facts will ultimately decide on the differences— but you might be surprised on how few there are that are not reducible to matters of expression. E.G., would a (hypothetical) F+ "vector" that comprised all the chromosomes [and might even be the F+ cell itself!] not be just a gamete. I agree with you that a public "confrontation" would have doubtful scientific value, but I would be happy enough to discuss these issues with anyone at any occasion of mutual convenience and appropriate atmosphere.

As you have no doubt been adequately indoctrinated with Hayes' point of view, I need not reinterpret this; you may be interested in my own (though this is fully, if rather obscurely) given in the paper in Genetics, Nov. 1952). That recombination is mediated by cell-to-cell contact is quite firmly established, at least by the negative results of all experimental efforts to the contrary. [Reference to factors extruded from corpses is picturesque language, but a speculation based on no facts; there are many examples of cells' retaining a mating capacity although they have been made vegetatively inviable. (It might be interesting to pursue this point with yeast, but one need perhaps refer only to the "lethal" effect of nutritional deficiency on the vegetative development of auxotrophic cells on minimal medium, although such cells are, of course, still capable of mating under the same conditions.) I am referring in this parenthesis, of course, to the experiments on the effects of streptomycin. Although the different reactivities of F- and F+ cells are only quantitatively different (since F+ cells are also progressive ly sterilized sexually by streptomycin) this is indeed very strong evidence for a physiological differentiation of these "mating types", and the finding is of course a very important contribution, though I suspect that Hayes has mis- or at least over-interpreted it.] As to the way in which the F+ acts to determine the compatibility phenotype, I have presently no very promising leads. It is not the sole determinant, for other genetic as well as environmental factors also play a decisive role. In fact, there are other (self-compatible) strains of E. coli in which the F agent seems to play no part whatever in compatibility, although it can be transferred to them as determined by infection and re-infection experiments. One must caution that the existence of an "F agent" is an epidemiological (or should I say epi-bacteriological) inference. We know there is an F+ quality, heritable within certain lines, and contagious to others, but it remains to be separated from the cells. On the whole, I would think that since recombination can be so readily demonstrated in cellular mixtures, and has not been detected otherwise, that the burden of affirmation rests on the claim for an additional, extra-cellular vehicle of recombination. Perhaps I am overly impressed by the ease with which, on the other hand, an extracellular vehicle is demonstrable in the Salmonella system, which also shows coordinate differences in genetic behavior. On the other hand, before we become too complacent about the operation of a sexual mechanism, it should be demonstrated morphologically (if it exists). We have had no reason to doubt that there were other than technical difficulties, and these may be partly averted by the discovery of the Hfr strains and the optimal conditions of recombination. We have, in fact, some leads in this direction as you may see from the enclosed photograph of HfrxF-. [This slide is over a year old, but until just now I have not been able to get involved more deeply in cytology. Please do not infer any claims of proof-- there are many possibilities of artifacts that will have to be disposed of.]

The genetic evidance is somewhat more fully developed. The hemizygosity of the Mal-S region in otherwise diploid isolates was, of cpurse, a disturbing puzzle. The first hypothesis considered was that some of the gametes were deficient, but had to be rejected in favor of the more complex notion of a post-zygotic elimination in the light of the following type of experiments. These have been expanded (largely by Tom Nelson) subsequent to the discovery of the compatibility situation:

In a cross of the form $M-\ Lac+\ Mal+\ F+\ \underline{Het}^{S^s}$ x $TL-\ Lac-\ Mal-\ F-\ ^{S^r}$, involving selection for M+.TL+ and subsequently Lac+/Lac- to be classified as diploids, about 85% of these are hemizygous $Mal-S^r$, and these cannot distinguish between a pre- and a post-zygotic elimination. However, 13% are $Mal+S^s$ and about 2% crossovers, either $Mal+S^r$ or, rarely, $Mal-S^s$. These classes are also hemizygous. Thus, 15% of the diploids are hemizygous for factors that have come from the F+ parent, and their <u>homolgues from the F-</u> have been eliminated. Mal+/- <u>heterozygotes</u> are simply not found in this material, although they have been synthesized by other means. The first critcism of this result would be that there had been in some way or other a reversal of polarity, so that the TL- parent had become F+ during the crossing, and subsequently crossed with phenotypically F- M-. However, tri-parental experiments have shown that illegitimate crossing of the two F- does not occur under the conditions of crossing when F+/// marked F-, F- and F+ are mixed. But even more important, the <u>crossover</u> hemizygotes have been eliminated in part from one parent, in part from the other. Our conclusion is that the elimination follows the opportunity for crossing-over in these cases. The assumption of a pre-zygotic elimination as well is not directly excluded, but is gratuitous. In more recent work, the polarity of F has been reversed, the parents remaining otherwise the same. We find here a comparable reversal in the incidence of Mal+ and Mal- (segmentally hemizygous) diploids. Most of the other markers, including Mtl and Xyl which are presumably on "unselected" chromosomes are regularly heterozygous. In our view, the discrepancies from random segregation of these markers from already well-established diploids (a discrepancy that may approach 10:1) not only would complicate a picture based solely on pre-zygotic irregularities of intact chromosomes, but are most readily accomodated in terms of their linkage to deficient segments. The function of F in all this is quite bizarre. We speculate that the chromosome(s) that has come from the parent which has functioned as the F+ parent is stigmatized so that it will later break at a certain point, and result in the loss of the distal segment. However, this follows crossing over, so that there is a 15% probability of recovering the F+ marker, corresponding to the inferred linkage distance of 15 units between the breakage point and the marker. In a few instances, these crossovers occur between Mal and S.

I do not want to take time to recite all of the evidence of this kind, involving other markers, and, for example crosses of diploid F+ x haploid F- (from which the issuance of diploid recombinants points to quite a large genetic content of the F+ gamete). In Salmonella, of course, we have found that only a single marker is ever transduced at any time with the unique exception that Bruce Stocker must have redited concerning the linkage of H_1:Fla_1. I will note one consequence of the Watson-Hayes theory that Tom Nelson pointed out has not been fulfilled in any of several adequate trials: the complementary crossover classes of linked pairs on unselected chromosomes should be equally frequent. Discrepancies of up to 10:1 have been noted and recorded in the literature (and rather facilely ascribed by us to linkage to post-eliminated segments or inclusion in them). Similarly, the segregation ratio of an "unselected chromosome" should be precisely reversed when the F polarity is reversed, and this is also definitely not the case.

I was pleased to hear of your work on the radiosensitive volume measurements of TP. Like yourself, I would not for a moment imagine that your stuff is phage. The Fluke addal, and your new findings do reinforce the view that transduction involves the transfer and implantation of a chromosome fragment, though this is of course not in any way inconsistent with its characterization as (principally) DNA. How about other size determinations? The sedimentation and electrophoresis measurements are of course useless with this kind of material, even for verification of purity, but I am rather surprised you had not long ago tried some gradocol filtratipns.

I am afraid I don't see the analogy between the new (and most interesting) story on the suppressive petites and "reduction" of prophage. May I ask, by the way, if there have been any really extensive attempts to reintroduce the normal plasmid* into petites by way of infection experiments? I bring this up because Rubbo is due to spend some months at the Enzyme Institute here, under the joint sponsorship of Perry Wilson, Dave Green and myself, and I had thpught to suggest that he undertake such attempts. The problem is of some interest here as the people at the Institute have spent considerable time in developing methods for the isolation of undamaged mitochrondria. However, whether Rubbo should be encouraged to spend his time along these lines will depend very much on Boris' comments, and how far you would be interested to cooperate by way of providing at least four cultures: the two wild type mating types, and the same in petites, though I assume there would be no difficulty in producing the latter by means of the published techniques.

Yours, with best wishes,

Joshua Lederberg

*pardon the expression.